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BACTERIAL AEROSOL OCCURRING IN ATMOSPHERIC AIR IN GLIWICE, UPPER SILESIA, POLAND

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Abstract
The aim of this work is to present the results of qualitative and quantitative analysis of airborne bacteria in the polluted urban atmosphere in Gliwice. The samples of airborne bacteria were collected using 6-stage Andersen cascade impactor (with aerodynamic cutoff diameters 7.0; 4.7; 3.3; 2.1; 1.1; and 0.65 µm). Additionally, the mean level of concentration of airborne solid particles was determined using the particle counter. The size distribution of the bacteria aerosol in winter and spring was also obtained. It has been found that the averaged airborne bacteria level in Gliwice in winter was 89 CFU/m^3 (geometric mean) and was elevated in spring more than five times. The main peak of the size-distributions in winter was located in the bacteria size range from 1.1 to 2.1 µm while in the spring the peak was shifted into larger bacterial particles. Concentration of coarse bacterial particles was highly correlated with the concentration of coarse particles of atmospheric particulate matter. The dominant groups of bacteria isolated from the atmospheric air in Gliwice were nonsporing Gram-positive rods.

Streszczenie
Celem pracy jest przedstawienie wyników analizy jakościowej i ilościowej bakterii zawieszonych w powietrzu zanieczyszczonej atmosfery miejskiej w Gliwicach. Próbki obecnych w powietrzu bakterii były pobierane przy pomocy 6-stopniowego impaktora kaskadowego Andersena (o średnicach odcięcia na poszczególnych stopniach wynoszących odpowiednio 7.0; 4.7; 3.3; 2.1; 1.1; oraz 0.65 µm). Ponadto, przy pomocy licznika cząstek, wyznaczono średnią wartość stężenia zawieszonych w powietrzu cząstek pyłu. Otrzymano także rozkłady ziarnowe aerozolu bakteryjnego w zimie i na wiosnę. Wykazano, że średni poziom bakterii w powietrzu Gliwic (średnia geometryczna) wynosił w zimie 89 CFU/m^3, a w lecie wzrósł ponad pięciokrotnie. W zimie główny pik stężeń rozkładu ziarnowego znajdował się w przedziale wielkości bakterii od 1.1 do 2.1 µm, a okresie wiosny był przesunięty w stronę większych cząstek bakteryjnych. Stężenie grubych cząstek bakteryjnych było silnie skorelowane ze stężeniem grubych cząstek pyłu w atmosferze. Dominującą grupą bakterii wyizolowanych z powierza atmosferycznego w Gliwicach były niezarodnikujące pałeczki Gram-dodatnie.

Keywords: **Bioaerosol; Airborne bacteria; Particulate matter; Atmospheric air.**

1. INTRODUCTION

It is well known that conducting a comprehensive monitoring of atmospheric aerosol concentrations is very important not only for the environmental management but also for the assessment of the health impact of air pollution. A special case of aerosol is biological aerosol, which is a two-phase system consisting of airborne biological particles. These particles, suspended in the air, may be viruses, bacteria, fungi or microscopic parts of living organisms. Bioaerosol's particles may be viable or non-viable. They occur as separate biological particles or are attached to the surface of dust created from inanimate matter. The smallest bio-particles aµmre viruses, the size of which varies from 0.02 µm to 0.3 µm. Bacteria and microscopic fungi cells are 0.3 to 100 µm in diameter and pollen and other biological particles from 10 µm up to even hundreds of micrometers [1-3]. Airborne bacteria and fungi can be the cause of a variety of infectious diseases as well as allergic and toxic. Infectious and non-infectious diseases caused by inhalation of different bioaerosols depend not only on the biological properties and chemical composition of these bioaerosols but also on the inhaled quantity and the site of their deposition in the respiratory tract. Because the deposition site in the respiratory tract of particles is directly related to the aerodynamic diameter of the particles, the health effect depends highly on their physical properties, and especially their size distribution.

The measurement of concentrations of biological aerosols is much more difficult than in case of dust aerosols. This applies especially to the living bacterial and fungal particles. Although the first brief collection of characteristics of some most popular bioaerosol samplers was made by Nevalainen et al. [4] twenty two years ago there are no suitable methods for the monitoring of the concentrations of living bacterial and fungal microorganisms in the air in real time. So far there are no automated methods and manual methods are time consuming. On the other hand, knowledge about concentrations of microorganisms for outdoor air is very poor. In particular, the dynamics of changes on the level of concentration of bacteria and microscopic fungi in atmospheric air in industrial areas is not sufficiently well understood. It appears that the air pollution present in these areas could severely modify the concentration levels of these bioaerosols, as well as their number size-distributions. This applies in particular to the interaction of bacterial aerosol with dust suspended in the air. It is estimated that about 80% of the microorganisms present in the air can be attached to dust particles, and lots of data points to strengthening of unfavorable, combined impact on the health of people exposed simultaneously to biological and dust aerosols [5, 6].

The aim of this study was to characterize the bacterial aerosols present in the atmospheric air in Gliwice, Upper Silesia, Poland, to guide future determination of some criteria for assessing Silesian outdoor air quality. It should be mentioned that in 2000 such studies of bacterial and fungal aerosol in indoor environment in Upper Silesia were carried out by Pastuszka et al. [7]. They found that the typical level of bacterial aerosol in Silesian homes is 10^3 CFU/m^3 and respirable bacteria represent 50% of the total. *Micrococcus* sp was the most frequently occurring bacteria in studied homes followed by *Staphylococcus epidermidis* which together contribute 50% of the total bacteria concentration.

This work concentrates on airborne bacteria only because these microorganisms are very sensitive during the interaction with other air pollutants and seem to be highly influenced by various meteorological factors: air temperature, wind speed, rain and snow, as well as solar radiation.

Because bacteria may attach to other particles ("rifting") and be transported with them [1, 8, 9], it turns out that in air with high concentration of particulates, the typical size distribution of bacterial aerosols can change. For this reason, the level of number concentration of airborne solid particles was also simultaneously determined using a particle counter.

2. METHODS

Measurements were carried out for bacterial aerosol concentrations in Gliwice with the simultaneous recording of meteorological parameters. As a method the impact method was used, which consisted of aspirator suction through a known volume of air at relatively high velocity striking the surface of agar media. Bacterial aerosol was collected on the height of 1.2 m using the 6-stage Andersen impactor. Moreover, the concentration was determined by means of quantitative dust particle counter. Andersen impactor consists of six stages (six impactors in series with the cut-off diameters, Dae, of 7.0, 4.7, 3.3, 2.1, 1.1, 0.65 µm), with increasingly lower inlet openings. The impactor is also composed of a pump, which provides a constant flow rate during measurement at 28.3 l/min. The measurement using the Andersen impactor lasted 10 minutes.

Microorganisms were drawn on the surface of nutrient agar in Petri dishes. The substrate suitable for the growth of the bacteria was TSA nutrient agar, to which cycloheximide (actidione) inhibiting the growth of fungi was added.

It is important to note that although a direct measurement of the concentration of airborne living bacteria is extremely difficult, the commonly used substitute for the concentration of living microorganisms present in the air is the number of Colony Forming Units in the volume of air CFU/m^3.

The next step was to identify the collected microorganisms, which took place in two stages. The first stage involved an analysis of morphological and microscopic colonies of grown cells stained with Gram. In the second stage API biochemical tests were carried out, which allowed the differentiation of the bacterial strains – on the basis of their metabolic properties.

In turn, the particle counter (Aero Track, USA) was used to measure the amount of particulate matter contained in the air volume. This counter was set for the quantitative measurement of the concentration of particles at the same time in six ranges: 7.0, 4.7, 3.3, 2.1, 1.1, and 0.65 μm. Particle counter measuring time was about 3.5 min.

3. RESULTS

The concentrations of bacterial aerosol in the atmospheric air (more precisely: outdoor air) in Gliwice are shown in Table 1.

Table 1.
Concentrations of the bacterial aerosol [CFU/m^3] (total bacteria) in the atmospheric air in Gliwice-Żerniki in winter and in spring 2013

	Winter	Spring
Number of sampling	N= 8	N= 19
Minimum	39	124
Maximum	217	3 244
Arithmetic Mean	102	669
Geometric Mean	89	462
Median	82	586
Standard deviation	60	709

It can be seen that the averaged airborne bacteria level in winter was 89 CFU/m^3 (geometric mean) while in spring 469 CFU/m^3, i.e. more than five times higher. Similar data were obtained in Taipei, Taiwan [10] and in Vienna [6] but the concentration level of airborne bacteria found in Beijing, China was about ten times higher than in Gliwice [11].

The highest concentration of bacteria in the atmospheric air in Gliwice was recorded in the spring, which was 3244 CFU/m^3. In contrast, the lowest concentration value amounting to 39 CFU/m^3 was recorded in the winter. However, continuing our study in summer we found significant decrease of the number of airborne bacteria able to grow as the colonies on the agar. The reason for this decline is, certainly, strong UV radiation of the sun in the summer. However, this part of the study needs continuation in the future.

Figure 1 and 2 shows the examples of the size distribution of bacterial aerosol in the atmospheric air in Gliwice. It can be seen that the size distributions in winter are two-modal while in spring one-modal.

It should be noted that for the winter data the main peaks of the studied size-distributions are located in the size range from 1.1 to 2.1 μm while in the spring there is one peak only, shifted into larger bacterial particles, and appearing between 3.3 and 4.7 μm of the aerodynamic diameter. This may indicate that during the moderate air temperature in spring, airborne bacteria can grow better in the surrounding environment (in soil, plants and in water) being, next, easily emitted into the air. It is also possible that airborne bacteria in the spring occurred most commonly in the form of bacterial aggregates and dust. This may be associated with high levels of particulate mat-

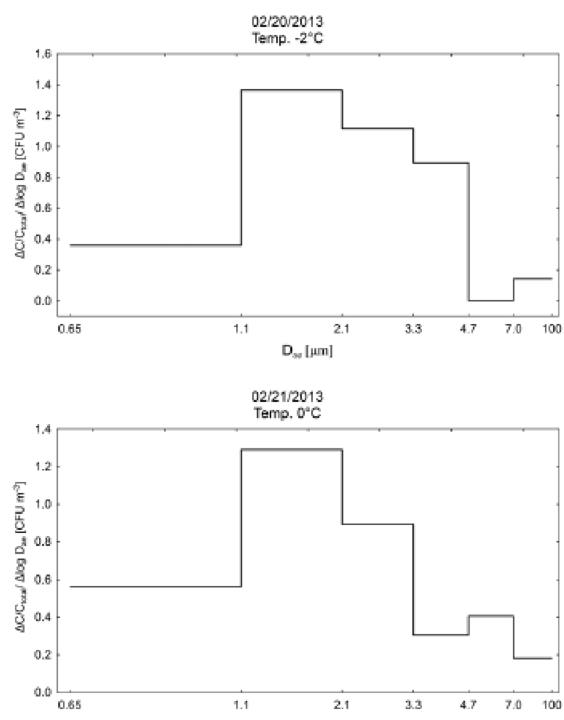

Figure 1.
Size distribution of the bacterial aerosol in the atmospheric air in Gliwice in winter

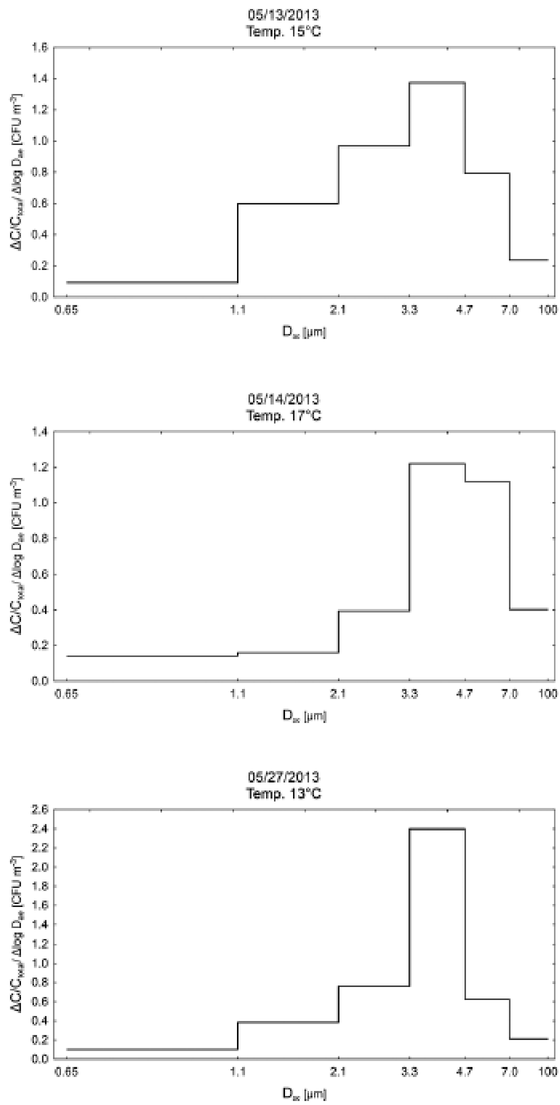

Figure 2.
Size distribution of the bacterial aerosol in the atmospheric air in Gliwice in spring

Table 2.
Viable bacterial genera identified in the atmospheric air in Gliwice in 2013

BACTERIA	Percent of the total bacterial genera [%] (Geometric Mean)	
	Spring	Winter
Gram-positive cocci,	**13**	**35**
including: *Kocuria rosea*	2	16
Micrococcus spp.	11	19
Staphylococcus xylosus	-	-
Nonsporing Gram-positive rods,	**31**	**53**
including: *Arthrobacter spp.*	-	11
Brevibacterium spp.	12	19
Corynebacterium auris	19	23
Sporing Gram-positive rods, family Bacillacae, including:	**28**	**7**
Bacillus pumilus	9	7
Bacillus cereus	19	-
Actinomycetes,	**18**	**5**
including: *Rhodococcus spp.*	18	3
Streptomyces spp.	-	2
Gram-negative rods,	**10**	-
including: *Pseudomonas spp.*	10	-

ter in the tested environment.

Important information about the exposure to atmospheric bacteria in Gliwice can be obtained from the detailed analysis of the bacteria genera and species.

As Table 2 shows the dominant groups of bacteria isolated from the atmospheric air in Gliwice in spring were nonsporing Gram-positive rods and Gram-positive endospore forming rods. The presence of Gram-negative rods in spring also should be noted. The main sources of these bacteria are soil (almost all Gram-negative airborne bacteria are of soil-origin), plants and water. In winter survival in the environment of bacteria family *Bacillacae*, *Actinomecytes*, and, first of all, the Gram-negative rods is very difficult, hence the dominating bacteria in this season is nonsporing Gram-positive rods, followed by Gram-positive cocci, constituting together 88% of the total bacteria genera.

Table 3 shows the number concentration of airborne solid particles measured simultaneously with the collection of airborne bacteria. It can be seen that in winter the concentration of particles in the atmospheric air was between 20 and 30 millions of particles in the cubic meter while in spring the mean concentration decreased almost 10 times (one order less). Certainly, the significant elevation of the airborne particles level in winter comparing to spring is related to the huge emission of particles from the coal combustion in the heating plants in Gliwice in winter season. It is interesting that this result agrees well with data obtained recently in Graz, Austria [6].

It is interesting to note that the ratio of the number of living bacteria to the number of solid particles in one cubic meter is approximately 10^{-4}. It means that the concentration of living bacteria in the atmospheric air is ten thousands lower than the concentration of solid particles larger than 0.65 µm.

Figure 3 shows a graph of the relation between the

Table 3.
Concentrations of the solid particles [m^{-3}] larger than 0.65 μm in the atmospheric air in Gliwice-Żerniki in winter and in spring 2013

	Winter	Spring
Number of sampling	N= 3	N= 8
Minimum	22.1 x 10^6	1.3 x 10^6
Maximum	30.7 x 10^6	25.8 x 10^6
Arithmetic Mean	25.6 x 10^6	6.3 x 10^6
Geometric Mean	25.3 x 10^6	3.7 x 10^6
Median	24.0 x 10^6	3.1 x 10^6
Standard deviation	4.5 x 10^6	8.8 x 10^6

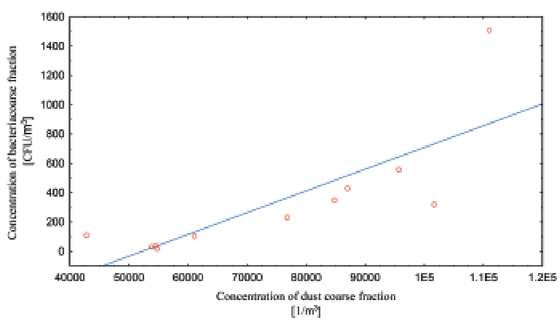

Figure 3.
Concentration of the coarse fraction of bacteria versus coarse solid particles (suspended dust) in the atmospheric air in Gliwice

concentrations of the coarse fraction of airborne dust (solid particles) and airborne bacteria, respectively, obtained in Gliwice.

The concept of the coarse fraction means here the solid particles or bacteria having the aerodynamic diameter d_{ae} > 3.3 μm. The equation of the regression line for the above graph can be assumed as follows y = -773.8212+0.0148 • x. At the significant level of 0.05, there is a strong correlation between the concentration of coarse bacteria and dust equal to 0.79.

It seems that the increase in the concentration of coarse airborne solid particles (dust) stimulates the increase in contribution of coarse bacterial particles in the total concentration of bacterial aerosol. This may be due to the adhesion of small, respirable bacteria (less than 3.3 μm) to the coarse dust particles suspended in the air. However, the linear increase of the concentration of coarse bacteria with increasing concentration of coarse solid particles (dust) can be related to the meteorological factors only. Such factors, like weak wind speed, lack of rain or snow, low altitude of mixing layer, are generally favorable for appearance of high concentration of any kind of coarse particles (dust and bacteria, fungi and so on) in the atmosphere. Therefore, the problem of the potential attachment of airborne bacteria to other particles in the polluted atmospheric air in Gliwice needs additional future studies.

4. CONCLUSIONS

The averaged airborne bacteria level in Gliwice in winter was 89 CFU/m^3 (geometric mean) and was elevated in spring more than five times reaching the level of 469 CFU/m^3.

The size distribution of bacteria aerosol appeared in winter as two-modal while in spring as one- modal.

The main peak of the size-distributions in winter was located in the size range from 1.1 to 2.1 μm while in the spring the peak shifted to larger bacterial particles, between 3.3 and 4.7 μm in the aerodynamic diameter.

The dominant groups of bacteria isolated from the atmospheric air in Gliwice were nonsporing Gram-positive rods. The second dominating bacteria species were sporing Gram-positive rods, family Bacillacae and Gram-positive cocci in spring and in winter, respectively. Gram-negative rods were also found in spring.

Concentration of living bacteria in the atmospheric air was ten thousands lower than the concentration of solid particles larger than 0.65 μm.

Concentration of coarse bacterial particles was highly correlated with the concentration of coarse particles of atmospheric particulate matter; however the mechanism responsible for this correlation needs future investigations.

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